

Nucleic Acid-Based Tools for Monitoring Bioremediation at Chlorinated Solvent Sites

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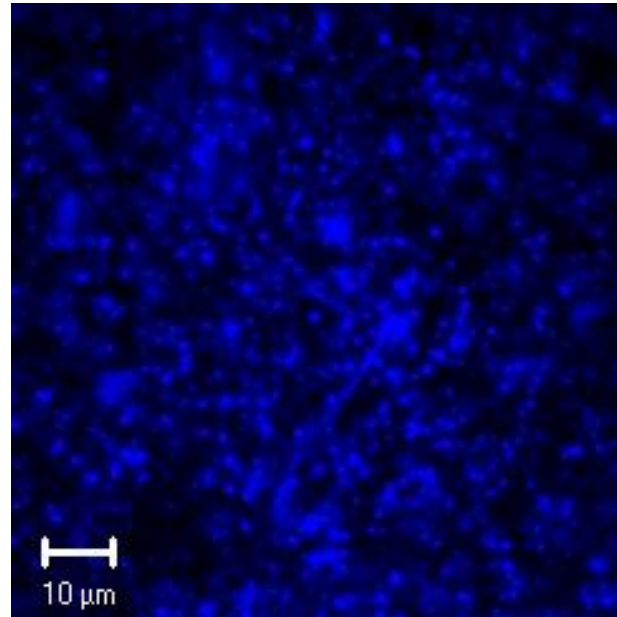
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What's In It For Me?

- **Learn the science behind MBTs**
- **Learn when to use MBTs**
- **Learn how to sample groundwater for MBTs**
- **Learn the “Rules of Thumb” for MBT data**
- **Learn how to save time and money with a smarter bioremediation design and operation– do I bioaugment?**

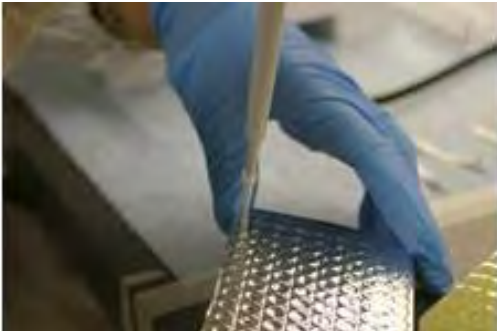


- **Nucleic acid probes (DNA or RNA)**
 - **Various targets including**
 - 16S rRNA gene
 - Functional genes (e.g., RDase, Hydrogenase, Oxygenase, etc.)
- **Protein biomarkers**
- **Lipid biomarkers**

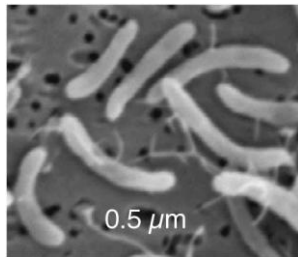
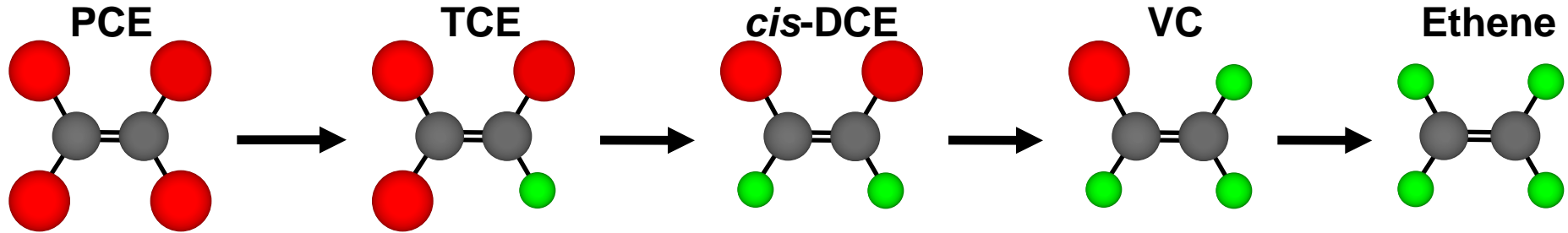


Purpose of MBTs

- **Reduce remediation costs and increase effectiveness by**
 - **Supporting sites where MNA is being evaluated**
 - **Predicting sites where biostimulation will succeed**
 - **Identifying sites where bioaugmentation is required**

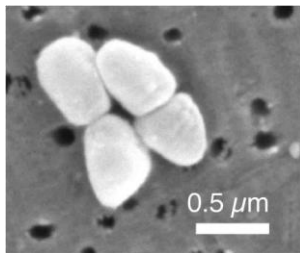


Chlorinated Ethene Reductive Dechlorination



Desulfitobacterium
sp. strain Viet1

Desulfitobacterium *sp.*
strain PCE1

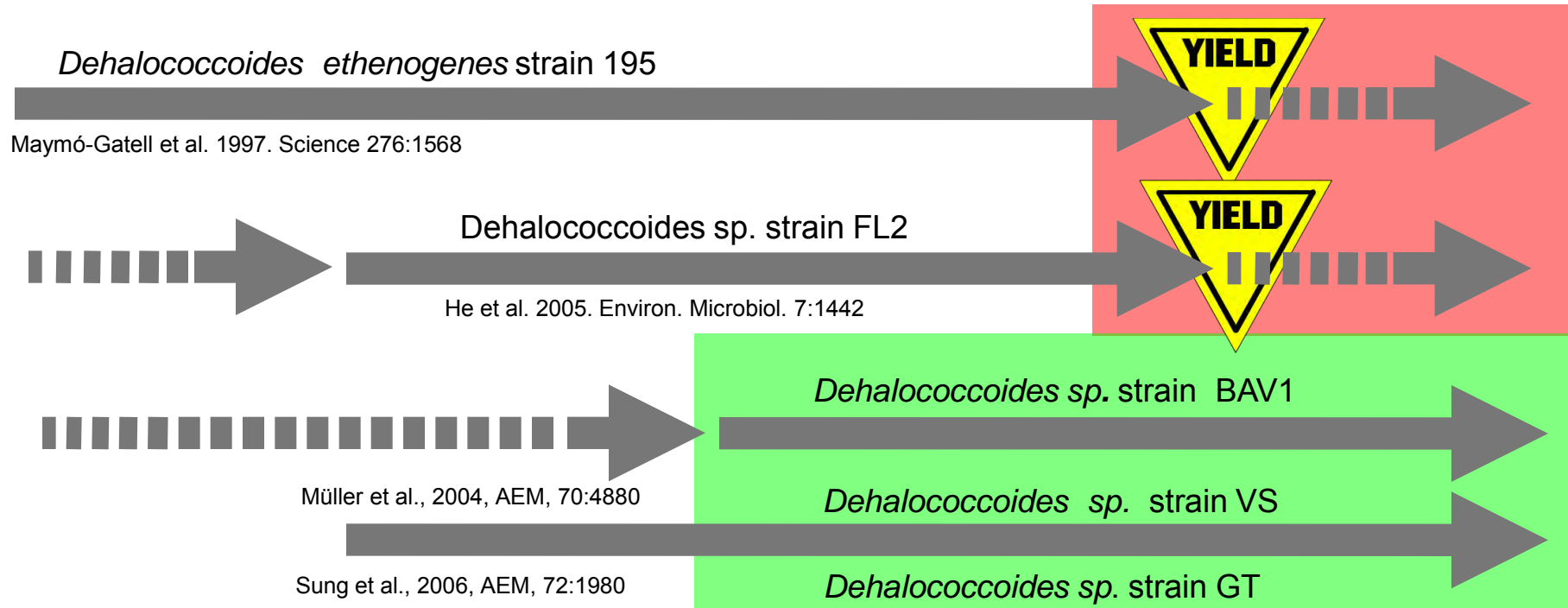
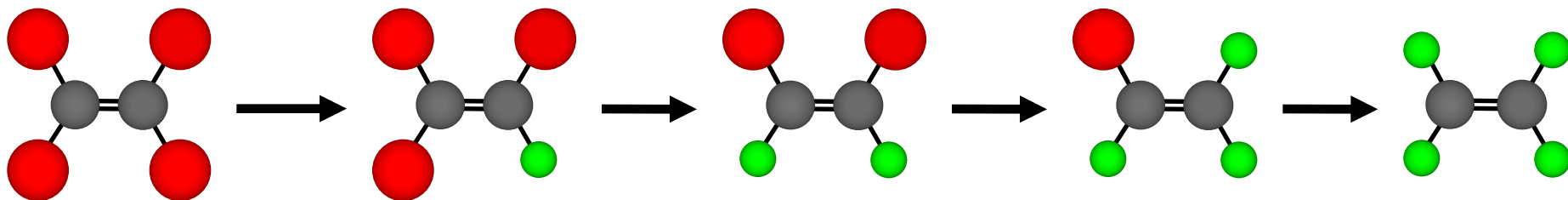


Desulfuromonas michiganensis

Sulfurospirillum,
Desulfitobacterium,
Dehalobacter, *Geobacter*



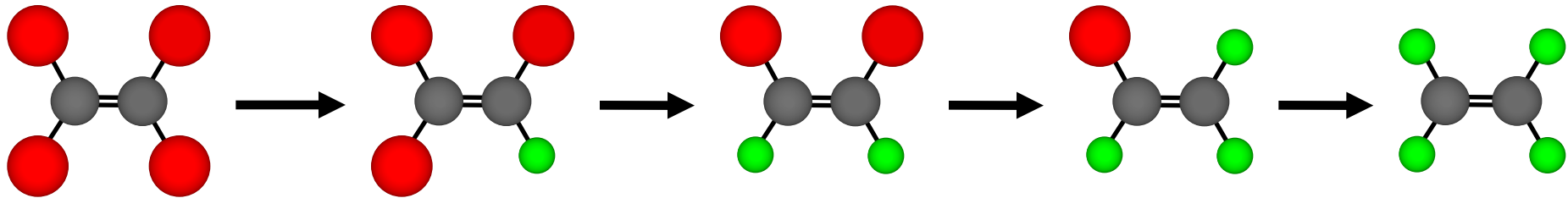
Dehalococcoides (Dhc) Involved in Reductive Dechlorination



- **16S rRNA found in all bacteria**
- **rRNA part of the ribosome; critical for protein biosynthesis**
- **Contains variable regions which allows for the differentiation of bacterial species**
- ***Dhc* has one 16S rRNA gene per cell**
- ***Dhc* 16S rRNA gene count = number of *Dhc* cells**

The 16S rRNA molecule has insufficient information to infer physiological traits

Dhc Reductive Dehalogenases



tceA



Dehalococcoides ethenogenes strain 195

Dehalococcoides sp. strain FL2

Dehalococcoides sp. strain BAV1

Dehalococcoides sp. strain VS

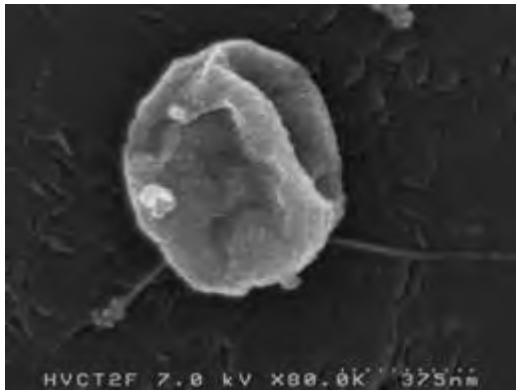
Dehalococcoides sp. strain GT

Dehalococcoides sp. strain KB-1/VS

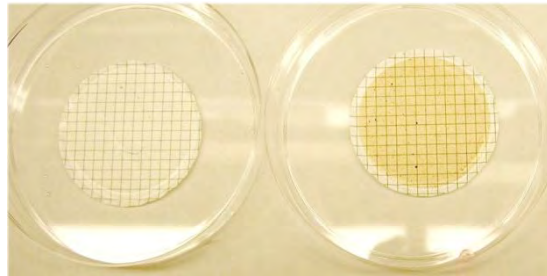
bvcA



vcrA



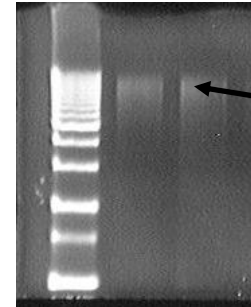
qPCR Sensitivity: Detection vs. Quantification



Extract Community DNA

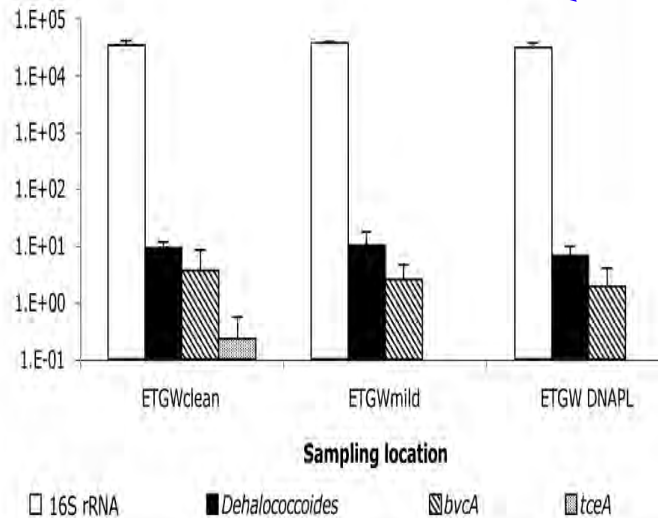
qPCR

PCR

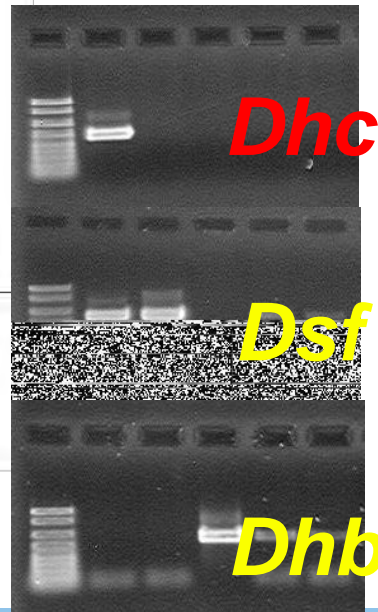


Genomic
DNA

Amplification with universal
16S rRNA gene-targeted
primers (for nested PCR)

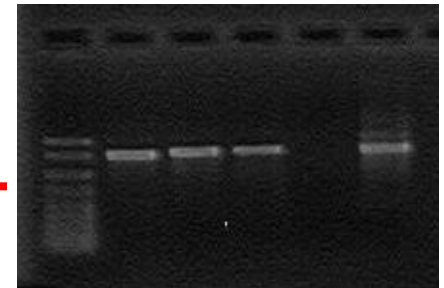


**Sensitive quantification
of dechlorinating bacteria
(~10³ copies/L)**



Dechlorinator
targeted primers

**Sensitive detection
of dechlorinating bacteria
(~10¹ copies/L)**



Dhc Rules of Thumb in the Field

<i>Dhc</i> 16S rRNA gene copies per L	Interpretation
10^3 or lower	Suboptimal <i>Dhc</i> to sustain dechlorination rates
$10^4 - 10^6$	May sustain appreciable dechlorination rates
10^7 or greater	Often associated with high rates of dechlorination and ethene production

- **Groundwater sampling preferred**
 - Difficulties with repetitive soil sampling
 - Spatial variability in soil
- **Sampling method influences results**
- **Use SOP that complements VOC sampling methods**



Groundwater Sampling

Shipping groundwater samples is problematic

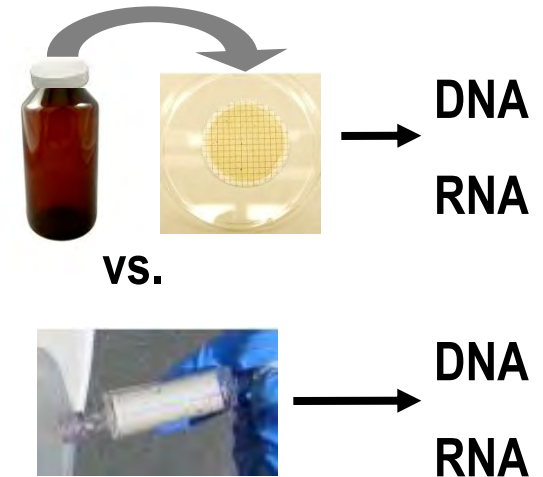
- Heavy, costly
- Leakage/breakage
- Biomarker integrity
- Groundwater disposal

Improved procedure: Filtration in the field

- Economical
- No leakage/breakage
- Biomarker stability?
- GW remains on-site



It is not practical to perform vacuum filtration in the field

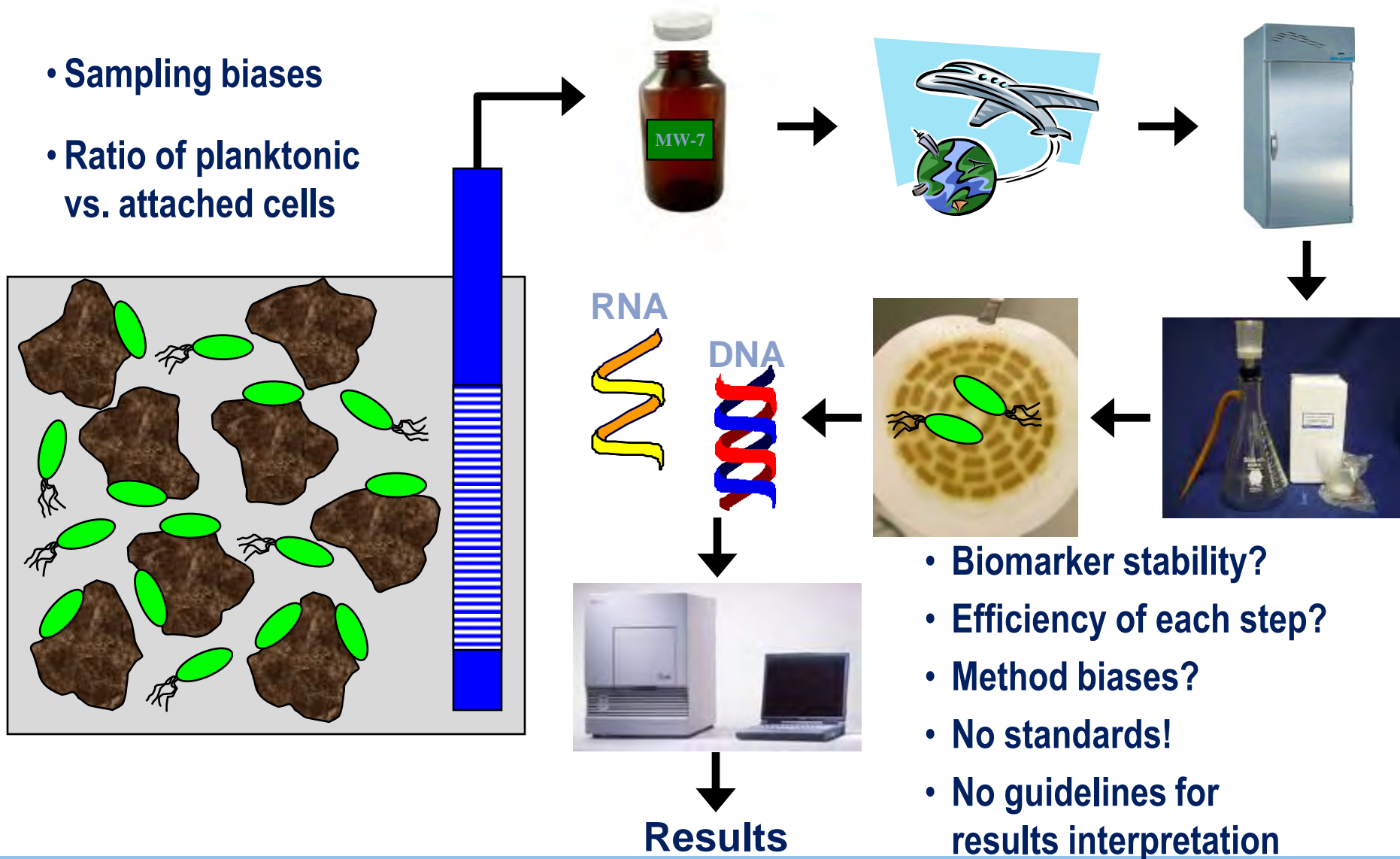


ESTCP Project ER-0518:

**Sterivex™ filters
are a viable alternative!**

Sampling Considerations

- Sampling biases
- Ratio of planktonic vs. attached cells

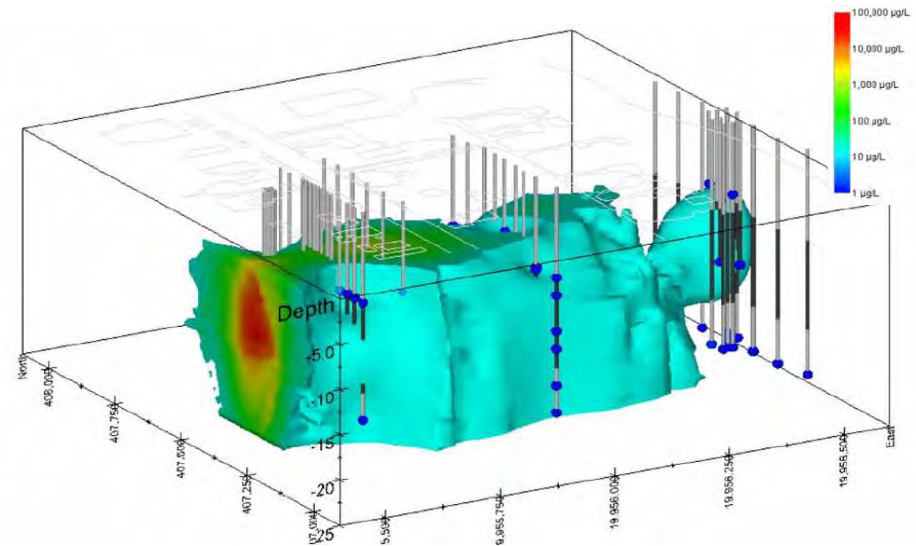


Groundwater Sampling SOP (ER-0518)

- **Low-flow purge**
 - Wait for stabilization of geochemical parameters to obtain a sample representative of formation groundwater
- **Surging**
 - Increases particulate matter in sample for recovery of associated (i.e., attached) biomass
- **Field filtration**
 - Sterivex™ filters for biomass collection in the field
 - Economical, no leakage/breakage, groundwater remains onsite
- **Shipping**
 - Secure samples for overnight shipment to laboratory
 - Maintain samples at 4°C

Sampling protocol should be defined and maintained for the duration of the monitoring effort for a particular site

- **Key sampling locations should include**
 - **Source area(s)**
 - **Downgradient plume locations where biodeg products observed**
 - **Vertical stratification**
 - Where possible, use discrete sampling zones and avoid sampling wells with extended screen intervals



- **Seasonal variability**
 - **Geochemical conditions and biomarker abundance can be affected by seasonal changes (e.g., rain events, temperature changes, etc.)...be aware!**
- **Bioremediation field implementation**
 - **Baseline and 1-2 months after injections**
 - **Quarterly in first 12-18 months**
 - **Collect with VOC, geochemical and TOC data**

- **Field labor**
 - Biomass collection can be performed concurrently with sampling events planned for assessment of contaminants
 - Minimal additional time is needed for collection of samples for biomarker analysis
- **Laboratory**
 - Microbiology labs specializing in biomarker analysis are typically independent from chemical laboratories used for other analyses
 - Typical cost for quantification of *Dehalococcoides* in a sample of groundwater is approximately \$250

- **Unnecessary bench tests**
- **Poorly designed pilot tests**
- **Inefficient full-scale treatment**
 - **Application of bioaugmentation and/or biostimulation when MNA would be appropriate**
 - **Bioaugmentation when sufficient Dhc are present to meet remediation goals**
 - **Failure to bioaugment when Dhc populations are insufficient**

- **TCE Source Area**
 - (~4,000 gal release 1960's)
- **Biostimulation**
 - Ethyl lactate
- **Performance monitoring**
 - Every other month
 - TCE, cDCE, VC, ethene
 - Dhc and vcrA
 - ~6 data points from each well

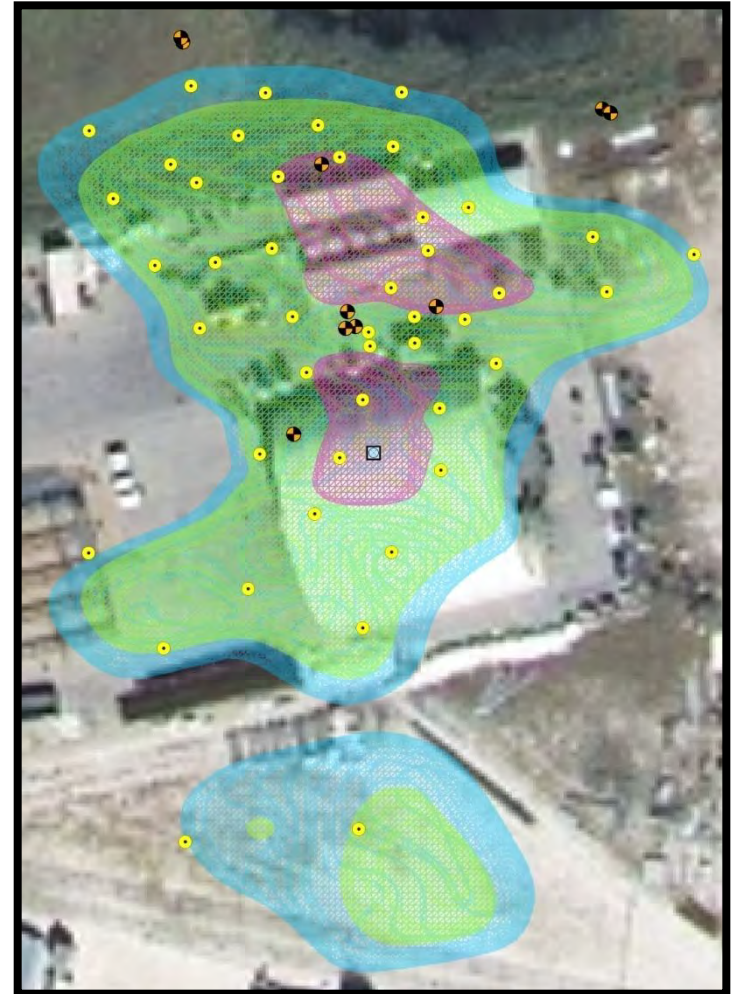
⦿ **Monitoring Well**

● **Injection Well**

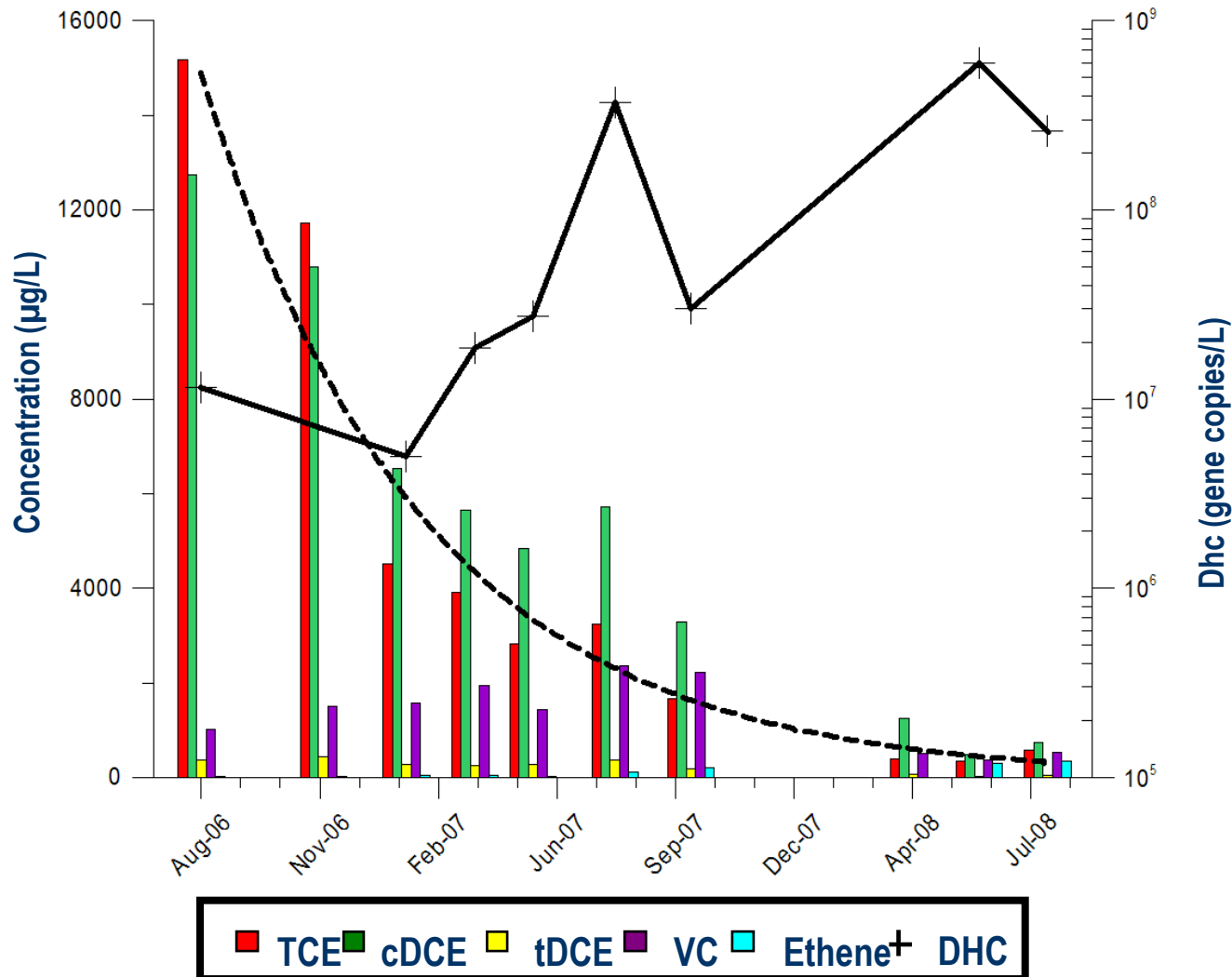
■ 30,000 µg/L TCE

■ 3,000 µg/L TCE

■ 1,000 µg/L TCE

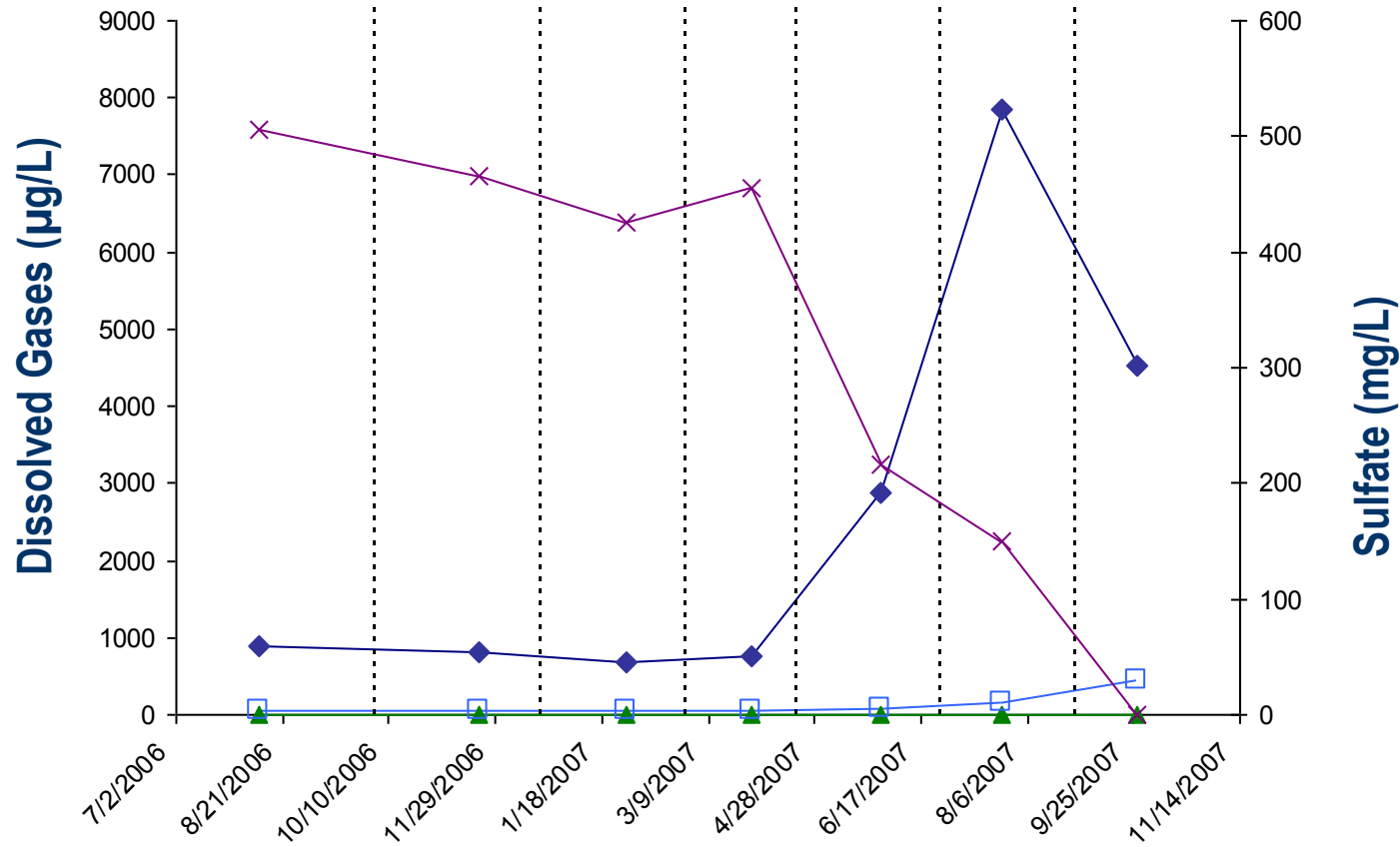


Groundwater VOCs and Dhc: SAMW-02



**Dhc data
indicated no
need to
bioaugment!**

Groundwater DHGs and Sulfate: SAMW-02

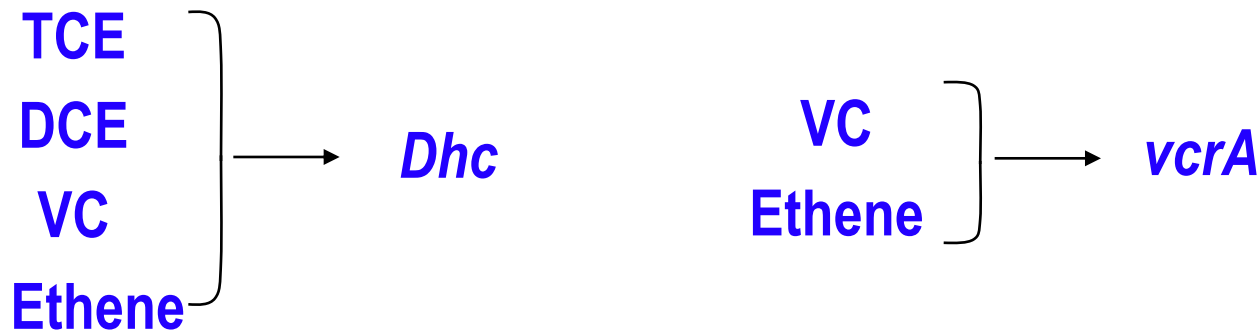


**Sulfate did not
inhibit
reductive
dechlorination!**

× Sulfate ▲ Ethane □ Ethene ◆ Methane --- Lactate injection

Correlation of *Dhc/vcrA* to VOCs and Ethene

Spearman Test



- **Correlation results:**

- *Dhc* or *vcrA* to TCE, DCE or VC

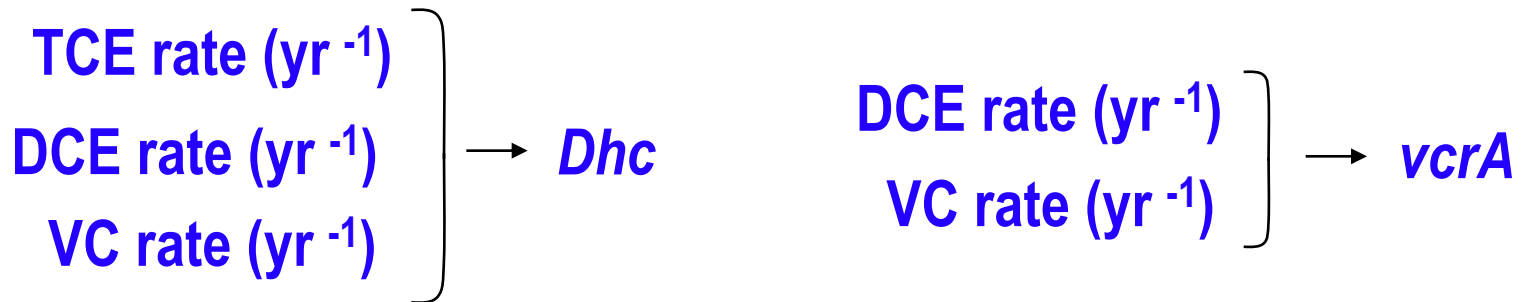
- Weak correlation ($r_s < 0.33$) for all comparisons

- *Dhc* or *vcrA* to ethene

- *Dhc* to ethene = strong correlation ($r_s = 0.66$; $n = 10$; $p = 0.05$)
 - *vcrA* to ethene = strong correlation ($r_s = 0.67$; $n = 10$; $p = 0.05$)

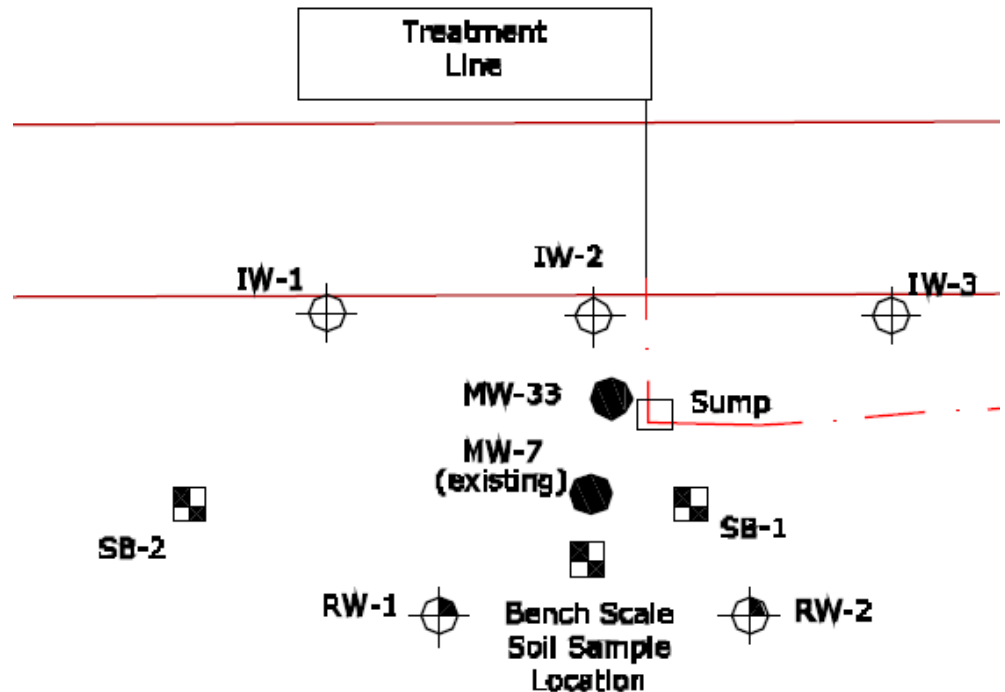
Correlation of *Dhc/vcrA* to Dechlorination Rates

Spearman Test

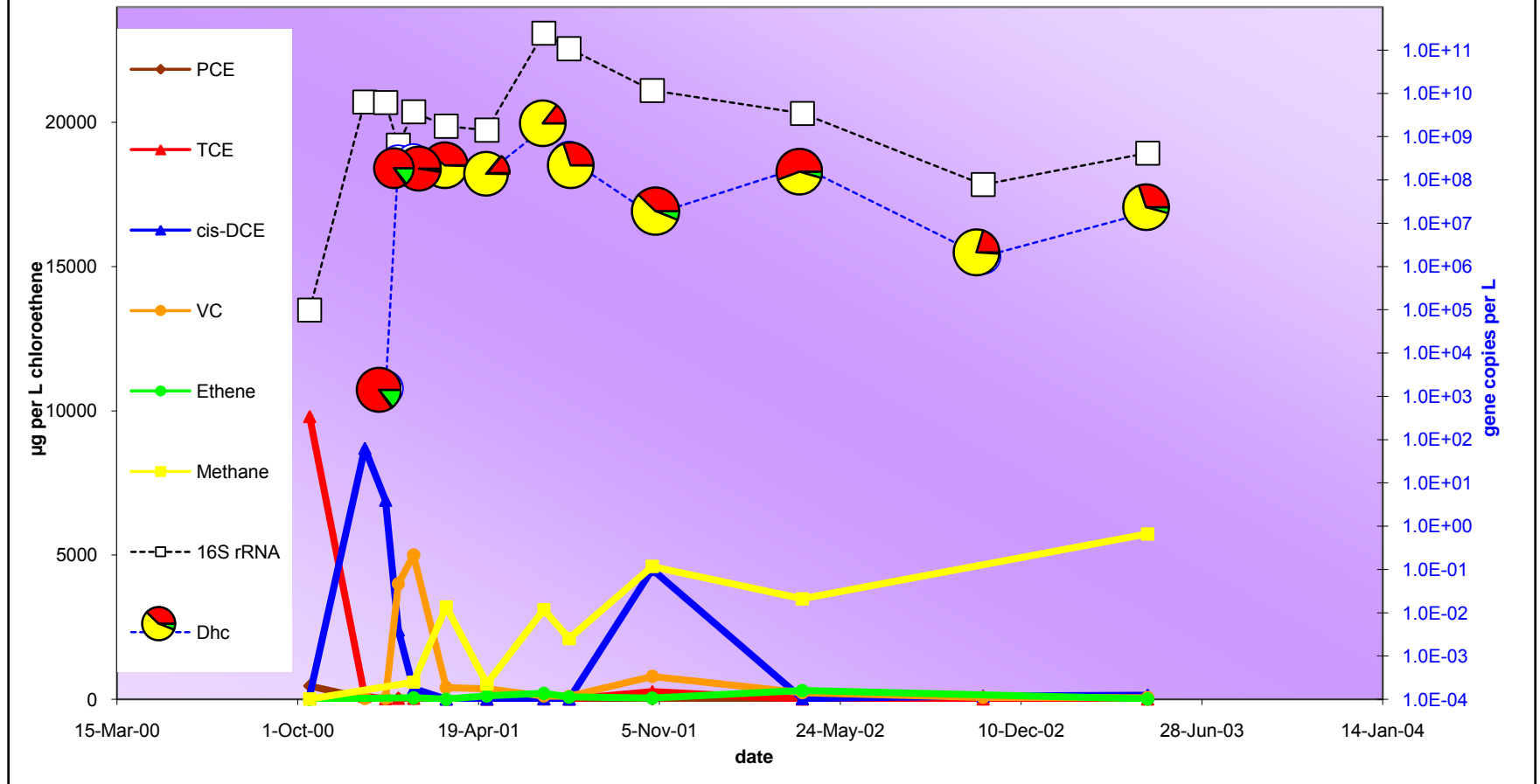


- Correlation results:
 - Strong correlations ($r_s = 1.00$) for all comparisons to *Dhc*
 - Medium correlations ($r_s = 0.50$) for all comparisons to *vcrA*
- Limited validity to results (only three data points)

- **TCE Source (18,000 ppb)**
- **Bioaugmentation Pilot Testing**
 - **3 injection wells and 2 recovery wells oriented perpendicular to the prevailing direction of groundwater flow (southwest)**
 - **Soluble electron donor (lactate) and dechlorinating culture distributed by recirc**

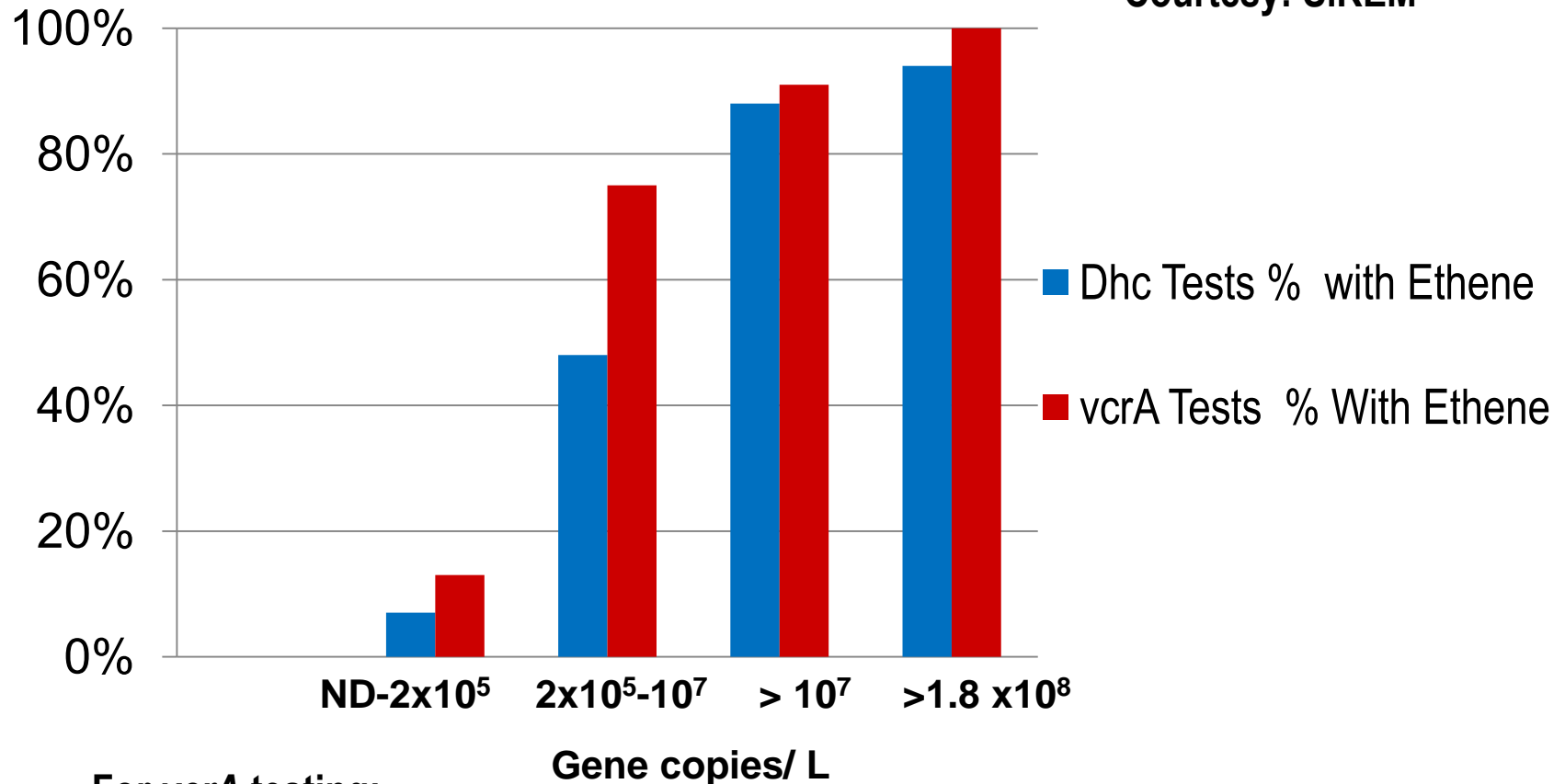


Dehalococcoides and chloroethenes



- ***Dhc* or *RDases* to VOCs**
 - No correlations to *Dhc*
 - Strong correlation of *bvcA* ($r_s = 0$, $n = 10$, $p = 0.02$)
 - Strong correlation of *vcrA* to cDCE ($r_s = -0.80$, $n = 10$, $p = 0.01$)
 - Strong correlation of *tceA* to VC ($r_s = 0.76$, $n = 10$, $p = 0.02$)
 - No other correlations identified

Courtesy: SiREM



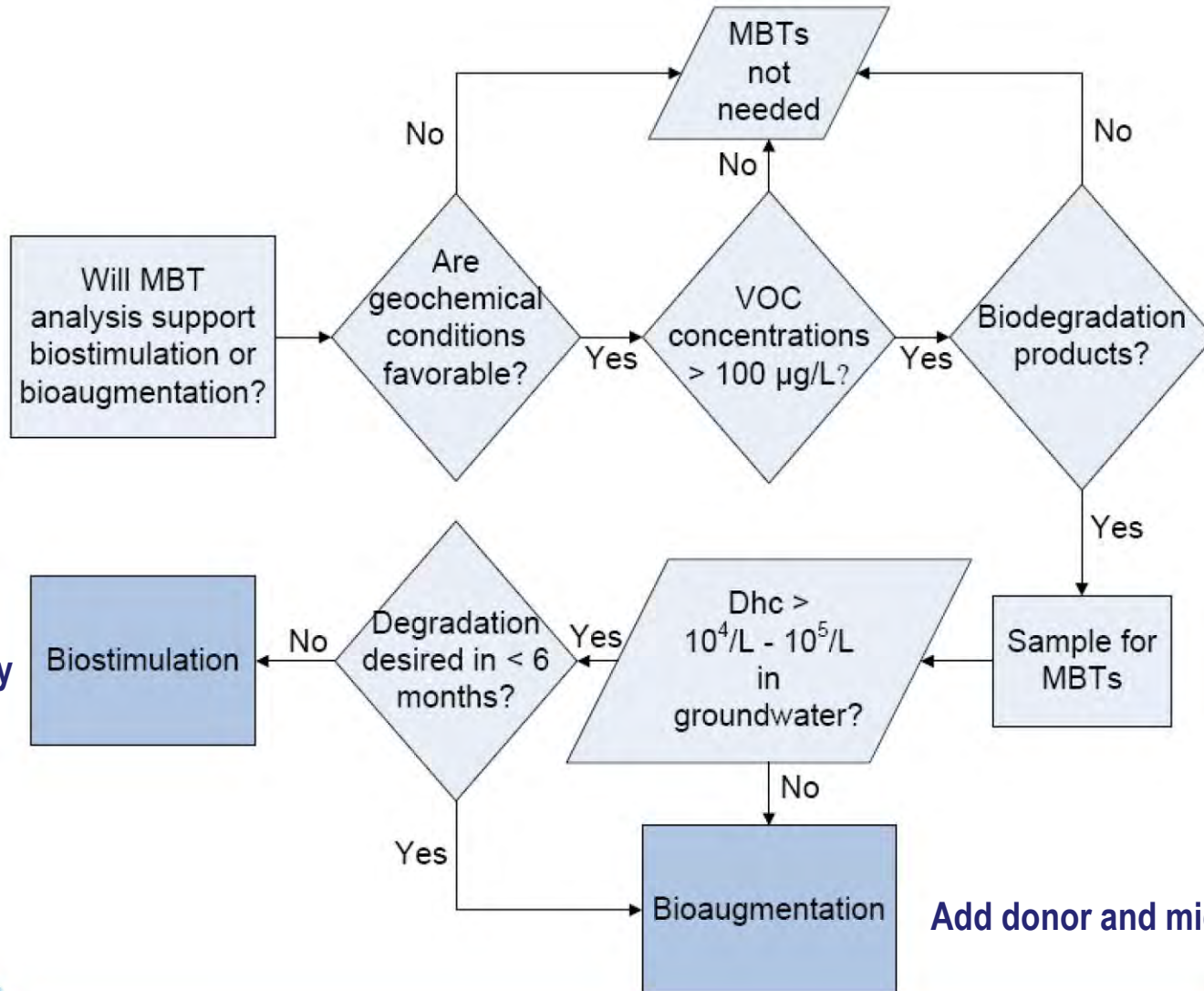
For *vcrA* testing:

- below 2 x 10⁵/L ethene not normally detected
- above 1 x 10⁷/L ethene commonly detected
- above 1.8 x 10⁸/L ethene always detected

N= 121 Samples *vcrA*

N=244 samples Dhc

Biostimulation/Bioaugmentation Flowchart



Add donor only

Add donor and microbes

Reminder-Dhc Rules of Thumb

<i>Dhc</i> 16S rRNA gene copies per L	Interpretation
10^3 or lower	Suboptimal <i>Dhc</i> to sustain dechlorination rates
$10^4 - 10^6$	May sustain appreciable dechlorination rates
10^7 or greater	Often associated with high rates of dechlorination and ethene production

- **MBTs are valuable tools to monitor biodegradation of chlorinated ethenes**
- **SOPs are available for MBT sampling. Field filtration is reliable.**
- **Biomarker genes (*bvcA*, *vcrA*) are indicators of field dechlorination activity**
- **Rules of thumb and draft guidance are available**
- **Understand limitations of the data**

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